POTENTIAL OF ALGAE OCCURING IN VARIOUS WATER BODIES OF AGRA, AS A SUSTAINABLE SOURCE OF BIOFUELS

A SYNOPSIS

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INTRODUCTION

Our petroleum reserves are limited resources. Now a days biomass energy technologies use waste or plant matters to produce energy with a lower level of green house gas emissions than fossil fuel sources (Sheehan *et al*. 1998).Further there is a increased stress a developing energy efficient technologies that can make biofuels more cost effective than fossil fuels (Puhan *et al*. 2005).

The world has entered a period of declining non-renewable energy resources, that known as 'peak oil' while energy demand is increasing the oil production is expected to decline in between one and ten decades (Crookes, 2006) as a result of energy critics, both governments as well as private industry are examining alternative sources of energy. There are other non-renewable sources of energy exist like coal and uranium, however, these source are limited and and will also inevitably decline in availability.

We depend on fossil fuels which caused CO_2 enrichment of the atmosphere, and it is primary contributor to the global warming. In order to realize a stable energy alternative source that will meet world demand while mitigating climate change, it is necessary to develop renewable clean fuels, so we are turning in direction of use biofuels (Schneider, 2006; Haag, 2006). Government organization and private sectors are taking seriously invest in the biofuels market (Scott and Bryner, 2006).

We are facing the frequent oil supply crisis, as a way to help nonfossil fuel producer countries to reduce energy dependence. The term biofuel is referred to solid, liquid or gaseous fuels derived from organic matter. They are divided into primary and secondary biofuels. The primary biofuel such as fuel wood is used in unprocessed from preferably for heating, cooking or electricity production, while secondary biofuels such as bioethanol and biodiesel are produced by processing biomass and are able to be used in vehicles and various industrial processes.

In present time we are focusing on biofuels because of many reasons to be considered as relevant technologies by both developing and industrialized countries (Demirbas, 2007),

including energy security reasons, environmental concerns, foreign exchange savings and socio-economic issues related to the rural sector because of its environmental merits, the share of biofuels in the automotive fuel market will grow fast in the next decades. There are many merits of consideration biofuels as a alternative source as following:

a. Biofuels are available from common biomass sources. b. they are represent a CO_2 cycle in combustion. c. they are environment friendly. d. there are many benefits the environment, economy and consumers in using biofuels. e. they are biodegradable and contribute to sustainability (Puppan, 2002).

There are two bio fuel which is replacement of gasoline and diesel fuel these are ethanol and biodiesel. Biomass based energy sources are carbon-di-oxide neutral and recycle the same carbon atoms, these are widespread available opportunities of biomass resources so biomass based fuel technology potentially employ more people rather than fossil fuel based technology (Kartha and Larson, 2000). There is rapid out growth of population and urbanization, as the result demand for energy is increasing everyday because of it the major conventional energy resources like coal, petroleum and natural gas are at the verge of getting extinct, biomass can be promising environment friendly renewable energy options.

Biofuels can be categorized into three generations: first, second and third generation biofuels. First generation biofuels includes bioethanol or butanol, by fermentation of starch (from wheat, barley, corn, potato) or sugars. Biodiesel by transesterification of oil crops. Second generation biofuels includes bioethanol and biodiesel produced from conventional technologies but based on novel starch, oil and sugar crops such as *Jatropha*, *Cassava.* Third generation biodiesel from microalgae, bioethanol from microalgae and seaweeds(Gupta *et al,*2012).

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novel starch, oil and sugar crops such as *Jatropha*, *Cassava.* Third generation biodiesel from microalgae, bioethanol from microalgae and seaweeds(Gupta *et al,*2012).

Microalgae have potential of convert CO_2 to biofuels, foods, feeds and high value bioactives so micro algae are called as sunlight driven cell factories (Metting and Pyne, 1986; Schwartz, 1990; Kay, 1991; Shimizu, 1996; Borowitzka, 1999; Walter *et al*. 2005), inspite of it, these photosynthetic microorganism are useful in bioremediation applications (Mallick, 2002; Suresh and Ravishankar, 2004) and also nitrogen fixing biofertilizers (Vaishampayan *et al*. 2001).

Many different type of renewable biofuels can provided by microalgae. Biodiesel derived from microalgae oil (Roessler *et al*. 1994; Sawayama *et al*. 1995 Gavrilescu and Chisti, 2005), and photobiologically produced biohydrogen (Ghirardi *et al*. 2000; Akkerman *et al*. 2002).

Using microalgae as a source of fuel, this idea is not new (Chisti, 1980-81; Nagle and Lemke, 1990) but now being taken seriously because of escalating price of petroleum and more significantly, the emerging issue concern about global warming which is associated with burning fossil fuels (Gavrilescu and Chisti, 2005) .Currently biodiesel is produced from plant and animal oils but not from microalgae but it is likely to change as several companies are attempting to commercialize microalgal biodiesel. Biodiesel is a proven fuel (Knothe *et al*. 1997; Fukuda *et al*. 2001; Barnwal and Sharma, 2005; Demirbas, 2005; Van Gerpen, 2005; Kulkarni and Dalai, 2006).

Algae have a number of unique advantage, algae do not require arable land for cultivation. So algae cultivation not need to compete with agricultural commodities for growing space. The water used in algae cultivation can be saline or fresh water (Brown and Zeiler, 1993). Algae have a greater capacity to absorb CO_2 than land plants, and also not prone to photosynthetic inhibition of intense sunlight (Brown and Zeiler, 1993).The cultivation of algae is performed in two ways: Open ponds and Bioreactors (Christi, 2007). Algae grow in aquatic environments, use light and CO_2 to create biomass. Algae are of two types macroalgae and microalge, macroalgae which are measured in inches and often seen growing in ponds. Microalgae are measured in micrometers and are tiny and normally grow in suspension within a body of water. Microalgae recognized as a good and potent

source for biofuel production because it has relatively high oil content and rapid biomass production.

Microalgae fastest growing photosynthesizing unicellular organisms and they can complete entire growing cycle in few days. Some species of algae have high oil content (up to 60% oil by weight and its can produce oil upto 15000 gallons per acre per year, under optimum condition) (Gupta *et al*. 2012). All algae possess green chlorophyll; algae masked by photosynthetic pigments that give them a distinguishing colour that is used to identify key division (Graham *et al*. 2008).

Algae can cultivate from open air ponds to closed photobioreactors with closely controlled environment (Graham *et al*. 2008). The range of optimal temperature for phytoplankton is within 20-30ºC, if temperature lowers than 16ºC will slow growth, and temperature high than 35ºC are normally adverse for a number of species (Andersen and Andersen, 2006). Algae can yield a wide variety of byproducts such as lipid, carbohydrate and proteins.

OBJECTIVES

TO KEEP THE IMPORTANCE OF BIOFUELS, THE PRESENT STUDY IS DESIGNED WITH THE FOLLOWING OBJECTIVES

1. To collect and identify locally available algae with respect to oil content.

2. Qualitative estimation and Quantitative (extraction) of algae present in abundance in various water bodies of Agra.

3. To mass cultivate oil rich algae.

4. To extract oil and Transestrify algal oil into biofuels.

5. To study fuel characteristics.

REVIEW OF LITERATURE

Schneider (2006) demonstrated that after oil extraction from algae, the remaining biomass fraction can be used as a high protein feed for livestock.

Sheehan *et al*. (2006) reported that algae are easy to cultivate, can grow with little or even no attention, using water unsuitable for human consumption and easy to obtain nutrients.

According to Chisti (2007) continued use of petroleum sourced fuels is now widely recognized as unsustainable because of depleting supplies and the contribution of these fuels to the accumulation of carbon dioxide in the environment. Biodiesel derived from oil crops is a potential renewable and carbon neutral alternative to petroleum fuels. unfortunately, biodiesel from oil crops, waste cooking oil and animal fat can not realistically satisfy even a small fraction of the existing demand for transport fuels. Microalgae appear to be the only source of renewable biodiesel that is capable of meeting the global demand for transport fuels, like plants, microalgae gratly exceeds the oil productivity of the best producing oil crops.

Campbell (2008) studied that biofuel derived from green algae biomass has the potential for high volume, cost effective production, it can be carbon neutral and produced intensively or relatively small area of marginal land.

According to Demirbas (2007) renewable energy sources are indigenous and can therefore contribute to reducing dependency on oil imports and increasing security of supply. The biofuel policy aims to promote the use in transport of fuels made from biomass, as well as other renewable fuels. Biofuels provide the prospect of new economic opportunities for people in rural areas and developing countries.

Evangelista *et al.* (2008) conducted that algae do not have distinct organs and structure that characterize land plants, such as leaves, roots etc.

Graham *et al*. (2008) observed that some algae are called as microalgae which is microscopic and able to float in water surface due to their lipid content called phytoplankton while some are

macroscopic and attach to rocks (Seaweeds). Size of algae may be less than the size of bacteria (0.5 μm) to over 50 m long.

Meng *et al*. (2009) reported that biofuel is an alternative fuel that can be produced from a variety of renewable sources. These sources include canola oil, soyabean oil, sunflower oil, cotton seed oil, animal fasts, and lipids produced by algae. Biodiesel in defined as a monoalkyl ester of a long chain fatty acid derived from the oils.

Cyanobacteria are autotrophic microalgae that require sunlight, CO_2 and nutrients for energy and carbon, the unique ability of the cyanobacteria cells to produce and secrete the target biofuels (Neiderholtmeyer *et al.* 2010).

Mata *et al*. (2010) reported that concerns have increased the interest in developing second generation biofuels produced from non food feed stocks such as microalgae which potentially offer greatest opportunities in the longer term.

Interest in algae as a feedstock for biofuels production has risen in recent years, due to projections that algae can produce lipids at a rate significantly higher than agriculture based feed stock (Weyer *et al*.2010).

Clarens *et al*. (2011) observed that use of algae make land more efficient rather than traditional biofuel crops and one of their advantages over these crops is than they do not require arable land.

Ananadhi and Stanleyreported that microalgae are more promising feed stock to their widespread availability and higher oil yields. Microalgal oil was extracted from *Chaetoceros* sp and physico- chemical properties were determined. The density, viscocity, acid value and free fatty acids were recorded and palmitic acid as major fatty acid.

According to Gupta *et al*. (2012) biofuel are considered to be the best way to reduce green house gas emission and alternative to pollutant fossil fuels. At present research is being conducted by culturing algae to produce different fuels like biodiesel, bioethanol etc. and using algae to produce biodiesel would be the only viable method to replace the need of gasoline used for automotive today.

According to Gupta *et al* (2012) microalgae are known to accumulate more lipids in nutrient deficient condition.

According to Gupta *et al*. (2012) microalgae contain lipid and fatty acids as membrane components, storage products metabolites and sources of energy. The lipid and fatty acid contents of microalgae vary in accordance with culture conditions.

As a sustainable source of energy, algae and the feedstocks, they produce great potential to meet the demands of replacing petroleum based fuels. The versatility of algae to produce lipids, carbohydrates, and proteins will be needed to crease multiple products in multiple markets to successfully satisfy economic demand (Menetrez, 2012).

Cagnon *et al*. (2013) studied that accumulation increased under the nitrogen replete condition. This study demonstrates that various types of oil mutants can be isolated in *Chlamydomonas***.**

Abdo *et al.* (2014) reported that isolation of high neutral lipid containing microalgae is a key for biofuels production. The florescent method has been successfully applied to the determination of lipids in certain microalgae. The cellular neutral lipids were determined and quantified using florescent Olympus microscope CKX41. Among the investigated sps. Which gave the highest lipid percentage were *Microcystis aeruginosa* and Chlamydomonas variabills gave 30% and 20% respectively.

PROPOSED METHODOLOGY

1. TO COLLECT AND IDENTIFY LOCALLY AVAILABLE ALGAE, WITH RESPECT TO OIL CONTENT.

For collection of algae, take 5 litre of plastic bottle for sample. Algal samples will be collected from various water bodies and identified same day of collection. For identification purpose PC compatible craft microscope will be used. To identify algae, take a drop of alga sample on a slide and seen under microsco(Lee *et al.*2014).

2. QUALITATIVE AND QUANTITATIVE (EXTRACTION) ESTIMATION OF ALGAE PRESENT IN ABUNDANCE IN VARIOUS WATER BODIES OF AGRA.

2.1 Qualitative estimation

There are some qualitative tests for the presence of lipids in a sample (Holland, 1999).

Translucency test:

This test involves a piece of filter paper and a hot plate and ether. Take the piece of filter paper and place a drop of the test solution on it. Then place the filter paper on the hot plate and heat to 60 for 5 minutes. Remove the filter paper and immerse in ether. After the paper is air dried, look at the spot. If the spot is translucent, there are lipids in the solution.

Sudan Red Test:

Sudan red is a lipid soluble dye. Add 2 ml of water in 2 ml of the test sample. Then add 5-6 drops of Sudan reagent (lipid soluble dye). Red colour of the sample confirms the presence of lipid in the sample.

2.2Quantitative (extraction) estimation

Quantitative estimation will be done by two methods

2.1.1 Mechanical method- It will be used for break of cell wall. In this method, algae will be pressed by ball mill and then extraction will be done by manual hydraulic press (Soni *et al.*2008).

2.1.2 Chemical method- In this method, soxhlet or clevanger apparatus with suitable solvent like n-hexane, Benzene and petroleum ether, will be used (Park *et al*. 2007).

3. TO MASS CULTIVATE OIL RICH ALGAE:

Algae can grow under varieties of conditions and environments. After qualitative and quantitative estimation of algae, we cultivate the oil rich algae. For mass cultivation of algae PVC (rectangular) tubs of 200 litre capacity will be used (Borowitzka,1999). Nutrient medium for blue green algae(BG-11) Rippka *et al.*1979), and for green algae (CCAP,1992).

3.1. HARVESTING AND DRYING OF OIL RICH ALGAE

When the culture reached at stationary phase, the biomass will harvest dried in hot air oven at 60 C. Many methods are adopted for harvesting like filtration, centrifugation, flotation, flocculation, etc. We will use filtration method for harvesting of algae(Borchard and Omelia,1961) and will also done manually by sieves/ nylon cloth. After harvesting it will dry, so algal flakes will found.

4. TO EXTRACT OIL AND TRANSESTRIFY OF ALGAL OIL INTO BIOFUELS:

4.1 EXTRACTION

The extraction process will be planned by a combination of two methods-

i. Mechanical Method - Drying and disruption of algae.

ii. Chemical Method- Extraction of algal oil by Soxhlet apparatus.

4.2 TRANSESTRIFICATION

The alga oil converts into biofuels by the reaction of transestrification. This is an equilibrium reaction where organic oil or complex triglyceride, can be processed into biofuel. In transesterification, fats or oils are reacted with an alcohol to produce esters and glycerol in a one step reaction. The catalyst (sodium hydroxide 1% used as catalyst) and alcohol are mixed together prior to the reaction with glycerides (Russel and Ronald, 2005). First the catalyst and

alcohol are mixed together. This mixture is then fed to a reactor, where it is then combined with raw oils and continuously stirred. Next, the mixture of glycerin, biodiesel, and unreacted methanol is fed to a separator. Biodiesel and methanol are separated from the glycerin byproduct by use of either density loops or gravity settling. Finally, biodiesel and methanol are purified through evaporation to allow for collection of pure biodiesel (Meher *et al*., 2006). The ester products can be used directly in diesel engines without blending it with petroleum diesel fuel.

Triglyceride + 3 Methanol ↔ Glycerine + 3 Methyl Esters (Biodiesel)

5. TO STUDY FUEL CHARACTERISTICS

To study fuel characteristics, we will use following techniques-

GC-Mass Spectroscopy and FTIR (Fourier transform infrared spectroscopy) by (Ananadhi *et al.* (2012).

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